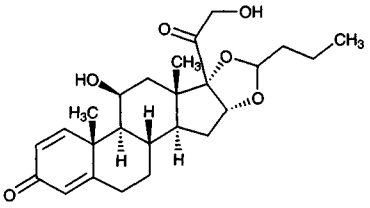
Budesonide



Molecular formula: $C_{25}H_{34}O_6$
Molecular weight: 430.54
CAS Registry No.: 51333-22-3 (β,16α), 51372-29-3 (11β,16α[R]), 51372-28-2 (11β, 16α[S])
Merck Index: 1490
Lednicer No.: 3 95

SAMPLE

Matrix: blood
Sample preparation: Add 5 mL diethyl ether/dichloromethane (ratio not given) to 1 mL serum, mix for 30 min. Centrifuge and freeze at -80° for 15 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue in 200 µL mobile phase. Inject a 50 µL aliquot.

HPLC VARIABLES

Column: Lichrospher RP Select B
Mobile phase: MeCN:20 mM ammonium acetate buffer 80:20
Flow rate: 1
Injection volume: 50
Detector: MS, PE-Sciex API 300, negative ion mode

CHROMATOGRAM

Internal standard: budesonide

OTHER SUBSTANCES

Extracted: flunisolide

KEY WORDS

serum; budesonide is IS

REFERENCE

Möllmann,H.; Derendorf,H.; Barth,J.; Meibohm,B.; Wagner,M.; Krieg,M.; Weisser,H.; Knöller,J.; Möllmann,A.; Hochhaus,G. Pharmacokinetic/pharmacodynamic evaluation of systemic effects of flunisolide after inhalation, *J.Clin.Pharmacol.*, **1997**, *37*, 893–903.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge twice with 3 mL portions of EtOH and twice with 3 mL portions of water. Add 1 mL EtOH:water 30:70 to 1 mL plasma, vortex, let stand for 15 min. Centrifuge at 1800 g for 15 min. Add the supernatant dropwise to the SPE cartridge, wash with 3 mL EtOH:water 25:75, 3 mL water, and twice with 2 mL ethyl acetate:n-heptane 2:98. Dry the SPE cartridge under vacuum, elute with 2 mL ethyl acetate:heptane 35:65. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Reconstitute the residue with 100 µL mobile phase, let stand 15 min. Inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 5 µm Hypersil ODS C18
Column: 100 × 2.1 5 µm Hypersil ODS
Mobile phase: EtOH:water 50:50
Flow rate: 0.45
Injection volume: 20
Detector: MS, Finnigan Mat TSQ 7000, ESI mode, positive ion APCI mode, m/z 473.2

CHROMATOGRAM
Retention time: 3.35 (budesonide acetate 22R epimer)
Internal standard: budesonide acetate 22R epimer

OTHER SUBSTANCES
Extracted: fluticasone

KEY WORDS
budesonide is IS; plasma; SPE

REFERENCE
Li,Y.N.; Tattam,B.N.; Brown,K.F.; Seale,J.P. A sensitive method for the quantification of fluticasone propionate in human plasma by high-performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 447–452.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg Extract Clean C18 SPE cartridge (Alltech) with two 3 mL portions of EtOH and two 3 mL portions of water. Add 50 μL 200 ng/mL IS solution and 1 mL EtOH:water 30:70 to 1 mL plasma, vortex carefully, let stand for 15 min, centrifuge at 1200 g for 20 min. Add the supernatant to the SPE cartridge attached to a vacuum manifold operating at 5.1 kPa, wash with 3 mL EtOH:water 25:75, 3 mL water, and 2 mL heptane:ethyl acetate 98:2. Elute with 2 mL heptane:ethyl acetate 65:35 and with 2 mL EtOH, evaporate the eluate to dryness under a stream of nitrogen at 35°, add 100 μL MeCN:triethylamine:acetic anhydride 75:12.5:12.5 to the residue, let react for 15 min, evaporate to dryness under a stream of nitrogen, reconstitute the residue with 100 μL mobile phase, let stand for at least 15 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm ODS Hypersil C18
Column: 100 × 2.1 5 μm ODS Hypersil C18
Mobile phase: EtOH:water 43:57
Flow rate: 0.5
Injection volume: 20
Detector: MS, Finnigan MAT TSQ 7000, APCI source, m/z 431, 476

CHROMATOGRAM
Retention time: 8.0 (22R), 9.0 (22S)
Internal standard: $^{2}H_{3}$ budesonide-21-acetate (preparation described in paper) (9-10.5)
Limit of detection: 700fg
Limit of quantitation: 250 pg/mL

KEY WORDS
derivatization; plasma; pharmacokinetics; SPE

REFERENCE
Li,Y.N.; Tattam,B.; Brown,K.F.; Seale,J.P. Determination of epimers 22R and 22S of budesonide in human plasma by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1996**, *683*, 259–268.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1.5 mL water + 4 mL dichloromethane, shake gently for 30 min, centrifuge at 2000 rpm for 15 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.9 10 μm Spherisorb ODS
Mobile phase: EtOH:water 40:60
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 7

KEY WORDS
plasma; dog; radiolabeled; pharmacokinetics

REFERENCE
Ryrfeldt,Å.; Tönnesson,M.; Nilsson,E.; Wikby,A. Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography, *J.Steroid Biochem.*, **1979**, *10*, 317–324.

SAMPLE
Matrix: blood
Sample preparation: 250-500 μL blood + 10 mL EtOH:10 mM acetic acid 10:90, mix, add 9 mL to a conditioned Sep-Pak C18 SPE cartridge, wash with 10 mL EtOH:10 mM acetic acid 8:92, elute with 4 mL EtOH:10 mM acetic acid 60:40. Dilute the eluate to an EtOH content of 8% and add it to another conditioned C18 SPE cartridge, wash with 10 mL EtOH:10 mM acetic acid 8:92, elute with 4 mL EtOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 μm Nucleosil NO2 + 200 × 5 5 μm Nucleosil NO2 in series
Mobile phase: dichloromethane:isopropanol:water 99:1:0.2 for 35 min then 75:25:0.2 for 15 min, re-equilibrate at initial conditions for 45 min
Flow rate: 3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 22-28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; pharmacokinetics; SPE

REFERENCE
Andersson,P.; Appelgren,L.-E.; Ryrfeldt,Å. Tissue distribution and fate of budesonide in the mouse, *Acta Pharmacol.Toxicol.(Copenh)*, **1986**, *59*, 392–402.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C18 SPE cartridge. Add plasma + IS to SPE cartridge, wash with aqueous EtOH, wash with water, wash with heptane, elute with ethyl acetate in heptane, esterify with acetic anhydride and triethylamine in MeCN, evaporate, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:100 mM pH 5 ammonium acetate 64:36
Flow rate: 1.4
Injection volume: 100
Detector: MS, Finnigan 4500 quadrupole, thermospray, scan time 40 ms, source block 220°, repeller 45 V, vaporizer 105°, jet block 180°, aerosol 220

CHROMATOGRAM
Retention time: 3.6 (as budesonide 21-acetate)
Internal standard: octadeutero budesonide
Limit of quantitation: 0.1 nM

KEY WORDS
plasma; LC-MS; SPE; derivatization

REFERENCE
Lindberg,C.; Paulson,J.; Blomqvist,A. Evaluation of an automated thermospray liquid chromatography-mass spectrometry system for quantitative use in bioanalytical chemistry, *J.Chromatogr.*, **1991**, *554*, 215–226.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2500 g for 10 min, mix the supernatant with an equal volume of 1 M pH 2.5 glycine buffer containing 0.2% Tween 20, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the supernatant on to column A and elute to waste with mobile phase A, after 1.4 min backflush the contents of column A on to column B with mobile phase B, after 3.6 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Flush column A with mobile phase A for 9 min and mobile phase B for 16 min.

HPLC VARIABLES
Column: A 30 × 2.1 Apex II aminopropyl (Jones Chromatography); B 50 × 2.1 Spherisorb C1 pH stable
Mobile phase: A 10 mM pH 2.5 glycine buffer containing 0.1% Tween 20; B Isopropanol: 10 mM pH 2.5 glycine buffer containing 0.1% Tween 20 3:97
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 22, 25 (epimers)

KEY WORDS
plasma; column-switching

REFERENCE
Lövgren,U.; Johansson,M.; Kronkvist,K.; Edholm,L.-E. Biocompatible sample pretreatment for immunochemical techniques using micellar liquid chromatography for separation of corticosteroids, *J.Chromatogr.B*, **1995**, *672*, 33–44.

SAMPLE
Matrix: blood, tissue
Sample preparation: Acidify plasma or lung tissue homogenate to pH 2 with 500 mM HCl, add 100 μL 20 μg/mL IS, extract with 8 mL dichloromethane. Evaporate the organic

layer to dryness under vacuum, reconstitute in 120 μL MeOH:5% acetic acid 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS C18
Mobile phase: MeCN:MeOH:water 44:11:45
Flow rate: 1
Injection volume: 80
Detector: UV 242 or radioactivity

CHROMATOGRAM
Internal standard: hydrocortisone 21-S-propionate (JO 498)

OTHER SUBSTANCES
Extracted: metabolites, beclomethasone dipropionate

KEY WORDS
plasma; rat; lung; radiolabeled; pharmacokinetics; epimers are separated

REFERENCE
Chanoine,F.; Grenot,C.; Heidmann,P.; Junien,J.L. Pharmacokinetics of butixocort 21-propionate, budesonide, and beclomethasone dipropionate in the rat after intratracheal, intravenous, and oral treatments, *Drug Metab.Dispos.*, **1991**, *19*, 546–553.

SAMPLE
Matrix: broncho-alveolar lavage fluid
Sample preparation: Centrifuge bronchoalveolar lavage fluid at 1150 g for 10 min. 1 mL Supernatant + 5 mL dichloromethane, shake on an alternating agitator for 10 min, centrifuge at 1150 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, centrifuge, inject an 80 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS C18
Mobile phase: MeOH:buffer 69:31 (Buffer was 0.1% acetic acid, pH 3.)
Flow rate: 1
Injection volume: 80
Detector: UV 250

CHROMATOGRAM
Retention time: 10.51
Limit of quantitation: 5 ng/mL

REFERENCE
Faouzi,M.A.; Dine,T.; Luyckx,M.; Brunet,C.; Gressier,B.; Cazin,M.; Wallaert,B. High-performance liquid chromatographic method for the determination of budesonide in bronchoalveolar lavage of asthmatic patients, *J.Chromatogr.B*, **1995**, *664*, 463–467.

SAMPLE
Matrix: bulk
Sample preparation: Inject a 5 μL aliquot of a solution in EtOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: EtOH:water 48:52 or 43:57
Flow rate: 1
Injection volume: 5

Detector: UV 254

CHROMATOGRAM
Retention time: 10, 11 (48:52), 18, 25 (43:57) (two epimers)

OTHER SUBSTANCES
Simultaneous: impurities, 16α-hydroxyprednisolone

REFERENCE
Roth,G.; Wikby,A.; Nilsson,L.; Thalén,A. High-performance liquid chromatographic determination of epimers, impurities, and content of the glucocorticoid budesonide and preparation of primary standard, *J.Pharm.Sci.*, **1980**, *69*, 766–770.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 µL of a solution of budesonide in MeOH with 625 µL 9% trifluoromethanesulfonic acid in MeOH, immediately add 625 µL 160 µm dansyl hydrazine in MeOH, after 12 h inject a 100 µL aliquot onto column A and elute to waste with mobile phase A, after 100 s backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. (Caution! Trifluoromethanesulfonic acid is highly toxic! Decontaminate surplus solutions with an equal volume of 2 M aqueous ammonia for 1 h!)

HPLC VARIABLES
Column: A 10 × 4.6 5 µm Vydac C18; B 150 × 4.6 5 µm Nucleosil C18
Mobile phase: A MeCN:7.7 mM pH 7 phosphate buffer 6:94; B MeCN:7.7 mM pH 7 phosphate buffer 65:35
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 520

CHROMATOGRAM
Retention time: 8, 10 (syn and anti isomers of 3-keto derivative)
Limit of detection: 1.5 pmole

KEY WORDS
derivatization; column-switching

REFERENCE
Hyytiäinen,M.; Appelblad,P.; Pontén,E.; Stigbrand,M.; Irgum,K.; Jaegfeldt,H. Trifluoromethanesulfonic acid as a catalyst for the formation of dansylhydrazone derivatives, *J.Chromatogr.A*, **1996**, *740*, 279–283.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue in ice cold saline for 30 s. 100 µL Homogenate + 4 mL EtOH:10 mM acetic acid 70:30, shake for 30 min, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 µL EtOH:water 30:70, add 5 mL 10 mM acetic acid, add to a conditioned Sep-Pak C18 SPE cartridge, wash with 5 mL 10 mM acetic acid, elute with 5 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute in 250 µL EtOH:water 30:70, inject a 200 µL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 µm Nucleosil C18
Mobile phase: EtOH:water 50:50 for 12 min then EtOH for 10 min
Flow rate: 1

Injection volume: 200
Detector: UV 254

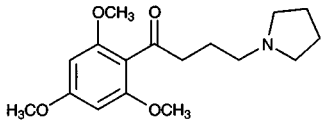
CHROMATOGRAM
Retention time: 11-13

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; pharmacokinetics; liver; lung; spleen; brain; SPE

REFERENCE
Andersson,P.; Appelgren,L.-E.; Ryrfeldt,Å. Tissue distribution and fate of budesonide in the mouse, *Acta Pharmacol.Toxicol.(Copenh)*, **1986**, *59*, 392–402.

Buflomedil



Molecular formula: $C_{17}H_{25}NO_4$
Molecular weight: 307.39
CAS Registry No.: 55837-25-7, 35543-24-9 (HCl)
Merck Index: 1498

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM

Retention time: 11.3

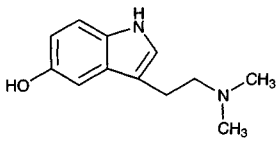
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Bufotenine



Molecular formula: $C_{12}H_{16}N_2O$
Molecular weight: 204.27
CAS Registry No.: 487-93-4
Merck Index: 1502

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

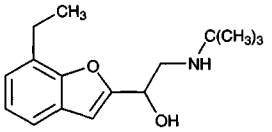
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine,

quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bufuralol



Molecular formula: $C_{16}H_{23}NO_2$, $C_{16}H_{23}NO_2$.HCl (hydrochloride)
Molecular weight: 261.36
CAS Registry No.: 54340-62-4, 59652-29-8 (HCl), 57704-10-6 ((-), HCl), 57704-11-7 ((+), HCl)
Merck Index: 1504

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 500 µL immobilized antibody (details of preparation in paper), wash with 5 mL 1 M NaCl, wash with 10 mL water, elute with 5 mL MeOH:10 mM pH 5.0 ammonium acetate buffer 95:5, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Ultron ES-OVM (Shinwa, Osaka, Japan)
Mobile phase: MeCN:0.3% pH 6.7 ammonium acetate buffer from 1:14 to 1:2 over 40 (?) min
Flow rate: 1
Detector: UV 248

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

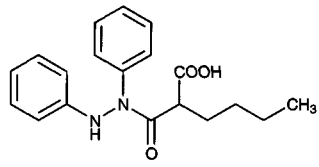
KEY WORDS

plasma; chiral separation of the metabolites but not necessarily of bufuralol

REFERENCE

Ikegawa,S.; Matsuura,K.; Sato,T.; Made,N.; Isriyanthi,R.; Niwa,T.; Miyairi,S.; Takashima,H.; Kawashima,Y.; Goto,J. Enantioselective immunoaffinity extraction for simultaneous determination of optically active bufuralol and its metabolites in human plasma by HPLC, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 1–9.

Bumadizon



Molecular formula: $C_{19}H_{22}N_2O_3$
Molecular weight: 326.40
CAS Registry No.: 3583-64-0, 69365-73-7 (calcium salt hemihydrate)
Merck Index: 1507

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 235

CHROMATOGRAM

Retention time: 6.72
Limit of detection: <120 ng/mL

KEY WORDS

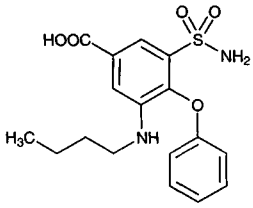
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Bumetanide



Molecular formula: $C_{17}H_{20}N_2O_5S$
Molecular weight: 364.42
CAS Registry No.: 28395-03-1
Merck Index: 1508
Lednicer No.: 2 87

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 30 µL 500 ng/mL methylbumetanide in water + 200 µL 1 M sulfuric acid + 6 mL diethyl ether, shake 20 min, centrifuge at 1000 g for 5 min. Evaporate 4 mL of ether layer at 30° under a stream of nitrogen. Dissolve residue in 150 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 22 × 3.9 37-50 µm Corasil C18
Column: 75 × 4.6 3 µm Supelco LC-8-DB
Mobile phase: MeCN:30 mM pH 3.0 sodium phosphate buffer 125:200
Flow rate: 1
Injection volume: 100
Detector: F ex 340 em 440

CHROMATOGRAM

Retention time: 4.5
Internal standard: methylbumetanide (6.5)
Limit of detection: 0.1 ng/mL

KEY WORDS

plasma

REFERENCE

Bökens,H.; Bourscheidt,C.; Müller,R.F. Determination of bumetanide in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *434*, 327–329.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 500 ng naproxen + 1 mL 100 mM HCl + 10 mL dichloromethane, extract. Dry organic layer at 50° under nitrogen, dissolve in 1 mL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 µm Alltech C8
Mobile phase: MeCN:80 mM phosphoric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: F ex 235 em 405

CHROMATOGRAM

Retention time: 12.3
Internal standard: naproxen (3.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: furosemide

KEY WORDS
plasma; horse; pharmacokinetics

REFERENCE
Singh,A.K.; McArdle,C.; Gordon,B.; Ashraf,M.; Granley,K. Simultaneous analysis of furosemide and bumetanide in horse plasma using high performance liquid chromatography, *Biomed.Chromatogr.*, **1989**, *3*, 262–265.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + piretanide + 1 mL MTBE + 100 μL 1 M pH 4 phosphate buffer, extract for 5 min, centrifuge. Remove 850 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 180 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:50 mM pH 3 NaH_2PO_4 30:70
Flow rate: 2
Injection volume: 50
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 6.2
Internal standard: piretanide (3.9)
Limit of detection: 0.4 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Oberbauer,R.; Krivanek,P.; Turnheim,K. Pharmacokinetics and pharmacodynamics of the diuretic bumetanide in the elderly, *Clin.Pharmacol.Ther.*, **1995**, *57*, 42–51.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 50 μL 250 μg/mL acetophenone in MeCN: water 50:50, vortex, add 400 μL MeCN, vortex, sonicate for 2 min, centrifuge for 10 min, inject an aliquot of the supernatant. Tissue. Homogenize (Tissuemizer) rat stomach, kidney, or liver with three volumes cold 250 mM sucrose, centrifuge at 9000 g. Incubate 1 mL supernatant with bumetanide, add 1 mL 1 M NaOH. 100 μL Mixture + 250 μL MeCN + 100 μL 0.3% HCl, vortex, centrifuge, inject an aliquot of the supernatant (from Biopharm. Drug Dispos. 1991, 12, 311).

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS-3
Mobile phase: MeOH:water:acetic acid 70:30:1
Flow rate: 1.5
Detector: F ex 338 em 433 or UV 254

CHROMATOGRAM
Retention time: 5.5 (F)
Internal standard: acetophenone (4.0, UV)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; human; rat; pharmacokinetics; stomach; kidney; liver; rabbit (J.Pharm.Sci. 1995; 84; 236)

REFERENCE
Smith,D.E. High-performance liquid chromatographic assay for bumetanide in plasma and urine, *J.Pharm.Sci.*, **1982**, *71*, 520–523.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 400 μL Serum + 100 μL 500 ng/mL IS + 1.0 mL 1.0 M pH 5.0 potassium citrate, vortex, let sit for 1 min. Add 4.0 mL ethyl acetate:cyclohexane 70:30, mix (Rototorque mixer) at 60 rpm for 10 min, centrifuge at 1150 g for 1 min. Evaporate upper organic layer to dryness under reduced pressure. Redissolve residue in 125 μL mobile phase, inject a 50 μL aliquot. Urine. Centrifuge sample at 16000 g for 1 min. Mix one volume urine supernatant with one volume 800 mM pH 3.9 potassium formate buffer, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 Zorbax C8
Mobile phase: MeOH:30 mM pH 2.5 potassium phosphate 63:37
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 340 em 440

CHROMATOGRAM
Retention time: 6.8
Internal standard: R021-1825 (6.3)
Limit of quantitation: 3 ng/mL (plasma), 10 ng/mL (urine)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Sullivan,J.E.; Witte,M.K.; Yamashita,T.S.; Myers,C.M.; Blumer,J.L. Pharmacokinetics of bumetanide in critically ill infants, *Clin.Pharmacol.Ther.*, **1996**, *60*, 405–413.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut 1 mL C18 SPE cartridge with 600 μL MeCN, 2 mL MeOH, and 3 mL water. 200 μL Plasma or urine + 20 (plasma) or 100 (urine) ng piretanide in methanol + 400 μL MeCN, vortex 2 min, sonicate 2 min in a water bath, centrifuge 10 min 1000 g. Transfer supernatant to a clean tube and add 4 mL 100 mM pH 5.0 phosphate buffer, add to SPE cartridge, wash with 3 mL water, elute with 300 μL MeCN. Evaporate to dryness under a stream of nitrogen at 37°, take up in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: C18 (unspecified)
Column: 100 × 8 5 μm C18 Radial Pak
Mobile phase: MeOH:water:glacial acetic acid 66:34:1
Flow rate: 1.2
Injection volume: 100
Detector: F ex 228 em 418

CHROMATOGRAM
Retention time: 6.5

Internal standard: piretanide (5.1)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: dopamine, amikacin, furosemide
Noninterfering: carbamazepine, phenytoin, midazolam, diazepam, lorazepam, ranitidine, gentamicin, erythromycin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, vancomycin, clindamycin, theophylline, digoxin
Interfering: nafcillin

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Wells,T.G.; Hendry,I.R.; Kearns,G.L. Measurement of bumetanide in plasma and urine by high-performance liquid chromatography and application to bumetanide disposition, *J.Chromatogr.*, **1991**, *570*, 235–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.055

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in mobile phase:acetone 95:5 containing 100 μg/mL salicylic acid, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Varian RP-8
Mobile phase: MeOH:water:acetic acid 60:40:0.5
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: 9.3
Internal standard: salicylic acid (4.5)
Limit of detection: 2000 ng/mL

KEY WORDS
tablets; ampoules

REFERENCE
Zivanov-Stakic,D.; Solomun,L.J.; Zivanovic,L.J. High-performance liquid chromatographic method for the determination of bumetanide in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1889–1892.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 228 em 418

CHROMATOGRAM
Retention time: 8.9
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: amiloride, furosemide, triamterene

KEY WORDS
column-switching; pharmacokinetics

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 100 μL water + 50 μL 500 μg/mL acetophenone in MeCN:water 50:50, vortex, inject an aliquot. (For rabbit (J.Pharm.Sci. 1995, 84, 236) or

rat (Biopharm. Drug Dispos. 1991, 12, 311) 100 μL urine + 250 μL MeCN, vortex, centrifuge, inject an aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS-3
Mobile phase: MeCN:15 mM phosphoric acid 50:50
Flow rate: 2
Injection volume: 6.5 (F)
Detector: F ex 338 em 433 or UV 254

CHROMATOGRAM
Internal standard: acetophenone (4.5, UV)

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
pharmacokinetics; human; rabbit; rat

REFERENCE
Smith,D.E. High-performance liquid chromatographic assay for bumetanide in plasma and urine, *J.Pharm.Sci.*, **1982**, *71*, 520–523.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM
Retention time: 9.3
Limit of quantitation: 1500 ng/mL

OTHER SUBSTANCES
Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, clopamide, methyclothiazide, furosemide, metolazone, mefruside, cyclopenthiazide, bendroflumethiazide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE
Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15.25 (A), 15.8 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

Interfering: ethacrynic acid

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 1 mL 10 mM HCl + 2000 ng bendroflumethiazide, extract with 5 mL ethyl acetate, centrifuge at 3000 rpm for 5 min. Remove the organic layer and dry it under a stream of nitrogen at 40°. Reconstitute with 100 μL MeOH, inject a 2 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: Gradient. MeOH: 50 mM ammonium acetate from 10:90 to 60:40 over 10 min, maintain at 60:40 for 10 min.

Column temperature: 40

Flow rate: 0.3

Injection volume: 2

Detector: F ex 231 em 426 or UV 230

CHROMATOGRAM

Retention time: 6.2

Internal standard: Bendroflumethiazide (F ex 223 em 415) (8.6)
Limit of detection: <10 ng/mL

OTHER SUBSTANCES
Extracted: furosemide (UV), piretanide (UV), cyclopenthiazide (UV), etozolin (UV), canrenone (UV)

REFERENCE
Gradeen,C.Y.; Billay,D.M.; Chan,S.C. Analysis of bumetanide in human urine by high-performance liquid chromatography with fluorescence detection and gas chromatography/mass spectrometry, *J.Anal.Toxicol.*, **1990**, *14*, 123–126.

SAMPLE
Matrix: urine
Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 3.7
Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES
Extracted: probenecid, ethacrynic acid, spironolactone

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 19.5

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85, inject a 20 μL aliquot. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid from 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min, maintain at 60:40 for 1 min, decrease to 10:90 over 1 min, equilibrate at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 7.2

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

Interfering: xipamide

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Inject 50 μL urine directly onto column A with mobile phase A and elute to waste, after 1 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 10
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene
Interfering: ethacrynic acid

KEY WORDS

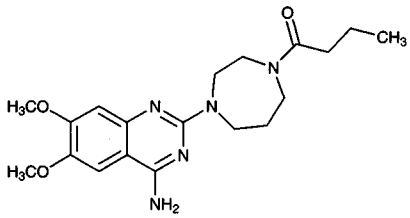
column-switching

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Bunazosin

Molecular formula: $C_{19}H_{27}N_5O_3$
Molecular weight: 373.46
CAS Registry No.: 80755-51-7
Merck Index: 1512



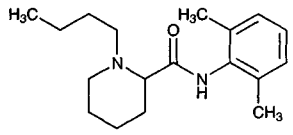
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:17 mM acetate containing 5 mM sodium laurylsulfate 50:50
Flow rate: 1
Detector: UV 245

KEY WORDS
water; buffer

REFERENCE
Kato,A.; Iwata,S. Studies on improved corneal permeability to bunazosin, *J.Pharmacobiodyn.*, **1988**, *11*, 330–334.

Bupivacaine



Molecular formula: $C_{18}H_{28}N_2O$
Molecular weight: 288.43
CAS Registry No.: 2180-92-9, 14252-80-3 (HCl hydrate), 18010-40-7 (HCl)
Merck Index: 1520
Lednicer No.: 1 17

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL water with 500 μL pH 6.0 buffer and 100 μL plasma sample, add 10 mL chloroform, shake mechanically for 10 min, centrifuge at 873 g for 10 min, evaporate the organic layer under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 35 μL aliquot. (Prepare pH 6.0 buffer by mixing 67 mM KH_2PO_4 with 67 mM Na_2HPO_4 in an 87.7:12.3 ratio.)

HPLC VARIABLES

Column: 250 × 2.1 5μm Supelcosil ABZ+plus deactivated reversed-phase
Mobile phase: MeOH:MeCN:50 mM pH 4.5 monobasic ammonium phosphate 5:7:63
Flow rate: 0.4
Injection volume: 35
Detector: UV 235

CHROMATOGRAM

Retention time: 26.90
Internal standard: bupivacaine (26.90)

OTHER SUBSTANCES

Extracted: cocaine, benzoylecgonine, norcocaine, cocaethylene,
Simultaneous: ascorbic acid, morphine, oxymorphone, noroxymorphone, norhydromorphone, norcodeine, codeine, nalorphine, procaine, acetaminophen, oxycodone, hydrocodone, caffeine, ethylmorphine, lidocaine, benzoylnorecgonine, ketamine, acepromazine, salicylic acid, benzoic acid, thebaine, cocaine propyl ester, benzocaine, tetracaine, pentobarbital

KEY WORDS

rat; plasma; pharmacokinetics; bupivacaine is IS

REFERENCE

Pan,W.; Hedaya,M.A. Sensitive and specific high-performance liquid chromatographic assay with ultraviolet detection for the determination of cocaine and its metabolites in rat plasma, *J.Chromatogr.B*, **1997**, *703*, 129–138.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 μL 2 μg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 μL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL of the organic phase to dryness under reduced pressure. Reconstitute the residue in 120 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 5 μm AGP bonded silica (ChromTech, Hagersten, Sweden)
Column: 150 × 4 5 micro.m AGP bonded silica (ChromTech, Hagersten, Sweden)

Mobile phase: Isopropanol:buffer 4:96 (Prepare mobile phase by adding 4% isopropanol and 0.6% diethylamine to 8 mM sodium dihydrogen phosphate containing 100 mM NaCl, adjust to pH 7.05 with 50% phosphoric acid.)
Flow rate: 0.9
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 29.35 (R-(+)), 38.25 (S-(-))
Internal standard: diazepam(19.21)
Limit of detection: 4 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine

KEY WORDS
plasma; pharmacokinetics; chiral

REFERENCE
Abraham,I.; Fawcett,J.P.; Kennedy,J.; Kumar,A.; Ledger,R. Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *703*, 203–208.

SAMPLE
Matrix: blood
Sample preparation: Directly inject 100 μL serum onto column A and elute to waste with mobile phase A, after 2 min elute to waste with mobile phase B, after 2 min backflush the contents of column A onto column B with mobile phase C, after 2 min remove column A from the circuit, elute column B with mobile phase C, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 4 min.

HPLC VARIABLES
Column: A 20 × 4.6 protein-coated Lichrosorb RP-8 (preparation details not given); B 250 × 4.6 Lichrosorb RP-18
Mobile phase: A pH 7.4 phosphate buffered saline; B MeOH:100 mM pH 5.5 phosphate buffer 20:80; C MeCN:MeOH:pH 6.4 phosphate buffer:ethylamine 30:30:40:0.3
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: <10
Limit of detection: 70 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, codeine, epinephrine, lignocaine, meperidine, morphine

KEY WORDS
serum; column-switching

REFERENCE
Emara,S.; Khedr,A.; Askal,H. Rapid and specific precolumn extraction high-performance liquid chromatographic assay for bupivacaine in human serum, *Biomed.Chromatogr.*, **1996**, *10*, 131–134.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL etidocaine hydrochloride in water + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM

Retention time: 8.7
Internal standard: etidocaine (12.0)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: 2,6-pipecolylxylidine, mepivacaine, lidocaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS

plasma

REFERENCE

Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 μg diazepam +100 μL 2 M NaOH + 7 mL n-hexane, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 Enantiopak α1-acid glycoprotein (LKB)
Mobile phase: Isopropanol:8 mM sodium phosphate buffer containing 100 mM NaCl 9:91
Column temperature: 30
Flow rate: 0.3
Injection volume: 20
Detector: UV 215

CHROMATOGRAM

Retention time: 21.0 (R), 28.3 (S)
Internal standard: diazepam (15.7)
Limit of quantitation: 500 ng/mL

KEY WORDS

serum; chiral

REFERENCE

Lee,E.J.; Ang,S.B.; Lee,T.L. Stereoselective high-performance liquid chromatographic assay for bupivacaine enantiomers, *J.Chromatogr.*, **1987**, *420*, 203–206.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 2 μg/mL etidocaine in water + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 20:80, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 12
Internal standard: etidocaine (10)
Limit of detection: 2 ng/mL

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 100 μg/mL lidocaine hydrochloride in water + 100 μL 2 M NaOH, mix, add 3 mL n-hexane, shake for 1 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and evaporate it to dryness with nitrogen under vacuum, reconstitute the residue in 200 μL mobile phase, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:50 mM pH 5.9 KH_2PO_4 38:62
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.9
Internal standard: lidocaine (3.8)
Limit of detection: 25 ng/mL

KEY WORDS
plasma

REFERENCE
Murillo,I.; Costa,J.; Salvá,P. Determination of bupivacaine in human plasma by HPLC, *J.Liq.Chromatogr.*, **1993**, *16*, 3509–3514.

SAMPLE
Matrix: blood

Sample preparation: Condition a Bakerbond cyano SPE cartridge two 1 mL aliquots of eluent. Add 2 mL MeCN to 1 mL plasma slowly while whirlmixing, let stand at room temperature for 5 min, centrifuge at 3000 g for 10 min. Remove the supernatant and dilute it with 15 mL water, add this mixture to the SPE cartridge, wash with three 1 mL aliquots of water, elute with eluent. Evaporate the eluent to about 500 μL under a stream of nitrogen, add 1 mL water, add 50 μL 1 M NaOH, whirlmix for 30 s, add 6 mL n-hexane, rotate for 10 min, centrifuge at 3000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 80 μL mobile phase, let stand for 2 h, inject a 50 μL aliquot. (The eluent was MeOH:50 mM NaH_2PO_4 adjusted to pH 3.0 with 1 M phosphoric acid 50:50.)

HPLC VARIABLES

Guard column: 10 × 4 Bakerbond chiral α1-acid glycoprotein
Column: 100 × 4 Bakerbond chiral α1-acid glycoprotein
Mobile phase: Isopropanol:10 mM pH 6.8 NaH_2PO_4 6:94
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 20 (R-(+)), 25 (S-(-))
Limit of detection: 10 ng/mL (S), 8 ng/mL (R)

KEY WORDS

plasma; SPE; chiral; pharmacokinetics

REFERENCE

Groen,K.; Zeijlmans,P.W.M.; Burm,A.G.L.; van Kleef,J.W. Improved clean-up procedure for the high-performance liquid chromatographic assay of bupivacaine enantiomers in human plasma and ultrafiltrate in the nanogram per milliliter range, *J.Chromatogr.B*, **1994**, *655*, 163–166.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL IS solution and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Prepare IS solution by adding 40 μL 1 mg/mL N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) in MeOH to 10 mL 100 mM NaH_2PO_4. Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM

Retention time: 7.2
Internal standard: N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) (14.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, mepivacaine, meperidine, fentanyl
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 2 M NaOH, vortex briefly, add 5 mL anhydrous ethyl ether, vortex for 30 s, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove 4.5 mL ether and add to 250 μL 12.5 mM sulfuric acid, vortex for 30 s, rotate for 10 min, centrifuge for 5 min, inject a 50 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Octyl 1B (Keystone)
Mobile phase: MeCN:50 mM Na_2HPO_4 27:73 pH adjusted to 5.8 with 50% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 9.18
Internal standard: bupivacaine

OTHER SUBSTANCES
Extracted: lidocaine, prilocaine, o-toluidine

KEY WORDS
plasma; pig; bupivacaine is IS

REFERENCE
Klein,J.; Fernandes,D.; Gazarian,M.; Kent,G.; Koren,G. Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite o-toluidine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 83–88.

SAMPLE
Matrix: blood
Sample preparation: 450 μL Plasma + 50 μL pentycaine in 10 mM pH 7 ACES buffer, vortex, filter (0.22 μm nylon syringe filter), inject a 10 μL aliquot onto column A with mobile phase A, after 3 min elute contents of column A onto column B with mobile phase B, monitor the effluent from column B. After 3 min remove column A from the circuit and re-equilibrate with mobile phase A. (ACES is N-(2-acetamido)-2-aminoethanesulfonic acid.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Regis semi-permeable surface (SPS) guard cartridge; B 100 × 3.2 7 μm Hypercarb pH (Shandon)
Mobile phase: A Isopropanol:buffer 3:97 (Buffer was 10 mM ACES adjusted to pH 7.0 with 5 M ammonia solution. ACES is N-(2-acetamido)-2-aminoethanesulfonic acid.); B 10 mM Acetic acid and 4 mM triethylamine in MeOH
Flow rate: A 0.8; B 0.5
Injection volume: 10

Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: pentycaine (6)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: ropivacaine

KEY WORDS
plasma; column switching

REFERENCE
Yu,Z.; Abdel-Rehim,M.; Westerlund,D. Determination of amide-type local anaesthetics by direct injection of plasma in a column-switching high-performance liquid chromatographic system using a pre-column with a semipermeable surface, *J.Chromatogr.B*, **1994**, *654*, 221–230.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of plasma to 7.4 with carbon dioxide gas. Filter (Amicon MPS-1 with YMT 30 membrane) while centrifuging at 37° at 500 g for 15 min, inject a 150 μL aliquot of the ultrafiltrate on to column A and elute to waste with mobile phase A, collect the eluate containing the compound in a 1 mL loop and inject it onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 125 × 4 4 μm Superspher RP-select B (Merck); B 150 × 4.6 5 μm Nucleosil 5 SA
Mobile phase: A MeCN:buffer 30:70 (Buffer was 3.1 mL 1 M phosphoric acid and 20 mL 1 M NaH_2PO_4 made up to 1 L with water, pH 3.); B MeCN:buffer 40:60 (Buffer was 149 mL 2 M ammonium hydroxide and 348 mL 1 M phosphoric acid made up to 1 L with water, pH 2.6.)
Column temperature: 27
Flow rate: 1
Injection volume: 150
Detector: UV 210

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: ropivacaine (elutes in a different fraction from column A)

KEY WORDS
plasma; ultrafiltrate; column-switching; rugged

REFERENCE
Arvidsson,T.; Eklund,E. Determination of free concentration of ropivacaine and bupivacaine in blood plasma by ultrafiltration and coupled-column liquid chromatography, *J.Chromatogr.B*, **1995**, *668*, 91–98.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Clean Screen SPE cartridge (Worldwide Monitoring) with two 2 mL portions of MeOH, with 3 mL water, and with 3 mL 10 mM pH 3.0

phosphate buffer. 1 mL Serum + 500 μL 10 mM pH 3.0 phosphate buffer, mix, add to the SPE cartridge, air dry for 30 s, wash with 3 mL phosphate buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with 2 mL chloroform:isopropanol:ammonium hydroxide 22:20.5:2.5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 SemiPermeable Surface (SPS) C8 (Regis)
Mobile phase: THF:2.5 mM potassium phosphate buffer 3.25:96.75 containing 0.0025% triethylamine, final pH adjusted to 2.7-2.8 with 85% orthophosphoric acid
Flow rate: 0.5
Detector: UV 235

CHROMATOGRAM

Retention time: 24
Internal standard: bupivacaine
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: acepromazine, atropine, benzoylecgonine, benzoylnorecgonine, cocaine, ketamine, norcocaine
Noninterfering: benzethonium chloride, benzyl alcohol

KEY WORDS

SPE; serum; bupivacaine is IS

REFERENCE

Muztar,J.; Chari,G.; Bhat,R.; Ramaro,S.; Vidyasagar,D. A high-performance liquid chromatographic procedure for the separation of cocaine and some of its metabolites from acepromazine, ketamine, and atropine from serum, *J.Liq.Chromatogr.*, **1995**, *18*, 2635–2645.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 10 μg/mL lidocaine in 25 mM sulfuric acid + 1 mL 1 M NaOH + 5 mL diethyl ether, shake or rotate for 15 min, centrifuge at 1000 rpm for 5 min, freeze at -20°. Remove the organic layer and add it to 250 μL 25 mM sulfuric acid, shake for 15 min, centrifuge at 1000 rpm for 5 min, freeze, discard the organic layer. Thaw the aqueous layer, pass air over the aqueous phase at room temperature to remove traces of ether, adjust pH to 5.0-6.5 by adding 10 μL 1 M NaOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Column: 125 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Mobile phase: MeCN:buffer 30:70 (Buffer was 7.0 g/L K_2HPO_4 in water adjusted to pH 5.8 with 1 M NaOH.)
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 202

CHROMATOGRAM

Retention time: 10
Internal standard: lidocaine (5)
Limit of quantitation: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Sattler,A.; Krämer,I.; Jage,J.; Vrana,S.; Kleemann,P.P.; Dick,W. Development of a HPLC-system for quantitative measurement of lidocaine and bupivacaine in patients plasma during postoperative epidural pain therapy, *Pharmazie*, **1995**, *50*, 741–744.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 264

CHROMATOGRAM

Retention time: 5.43

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; op-

ipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 4 mL MeOH and 4 mL water. 1 mL Serum or 300-500 μL ultrafiltrate + lidocaine, add to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 5:95, wash with 2 mL EtOH:water 2.5:97.5, wash with 2 mL MeCN:water 10:90, elute with 1 mL MeCN:50 mM pH 2.4 phosphate buffer 25:75, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 4.6 μBondapak
Mobile phase: MeCN:50 mM pH 4.0 KH_2PO_4 25:75
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Internal standard: lidocaine
Limit of detection: 10-15 ng/mL

KEY WORDS

serum; ultrafiltrate; SPE

REFERENCE

Mazoit,J.X.; Cao,L.S.; Samii,K. Binding of bupivacaine to human serum proteins, isolated albumin and isolated α-1-acid glycopotein. Differences between the two enantiomers are partly due to cooperativity, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 109–115.

SAMPLE

Matrix: blood

Sample preparation: Filter (0.22 μm) plasma, add ropivacaine (590 ng per 1 mL plasma), inject a 500 μL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 7 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 C18-alkyl-diol silica (J.Chromatogr.B 1995, 666, 315; J.Pharm.Biomed.Anal. 1995, 13, 615); B 10 × 4.6 5 μm Kromasil C8 (Eka Nobel) + 100 × 4.6 5 μm Kromasil C18 (Eka Nobel)
Mobile phase: A Isopropanol:buffer 5:95; B MeOH:buffer 63:37 (Prepare buffer by mixing 36.68 mL 1 M phosphoric acid with water, adjust pH to 7.4 with 1 M NaOH, make up to 2 L with water.)
Column temperature: 30 (column B)
Flow rate: A 1.5; B 1
Injection volume: 500

Detector: UV 210

CHROMATOGRAM
Retention time: 15
Internal standard: ropivacaine (9)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Yu,Z.; Westerlund,D. Direct injection of large volumes of plasma in a column-switching system for the analysis of local anaesthetics. II. Determination of bupivacaine in human plasma with an alkyl-diol silica precolumn, *J.Chromatogr.A*, **1996**, *725*, 149–155.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm) plasma, inject a 500 μL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 10 5 μm semi-permeable surface C8 (Regis); B 10 × 4.6 5 μm Kromasil C8 (Eka Nobel) + 100 × 4.6 5 μm Kromasil C18 (Eka Nobel)
Mobile phase: A Isopropanol:buffer 5:95; B MeOH:buffer 63:37 (Prepare buffer by mixing 36.68 mL 1 M phosphoric acid with 68.34 mL 1 M NaOH and diluting to 2 L with water, pH 7.7.)
Flow rate: A 1.5; B 1
Injection volume: 500
Detector: UV 240

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Extracted: ropivacaine

KEY WORDS
plasma; column-switching

REFERENCE
Yu,Z.; Westerlund,D. Direct injection of large volumes of plasma in a column-switching system for the analysis of local anaesthetics. I. Optimization of semi-permeable surface precolumns in the system and characterization of some interference peaks, *J.Chromatogr.A*, **1996**, *725*, 137–147.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 200 μL 2 μg/mL etidocaine in water + 1 mL 100 mM pH 9.0 sodium tetraborate + 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and add it to 500 μL 200 mM HCl, shake for 20 min, centrifuge at 1200 g. Remove the aqueous layer and add it to 1 mL 100 mM pH 9.0 sodium tetraborate, add 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 50°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nucleosil C8

Mobile phase: THF:10 mM pH 2.4 potassium phosphate 8:92
Flow rate: 1.6
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 4.15
Internal standard: etidocaine (10.60)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Lindberg,R.L.P.; Kanto,J.H.; Pihlajamäki,K.K. Simultaneous determination of bupivacaine and its two metabolites, desbutyl- and 4'-hydroxybupivacaine, in human serum and urine, *J.Chromatogr.*, **1986**, *383*, 357–364.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL 5 μg/mL IS in water + 100 μL 1 M pH 10 sodium carbonate buffer, shake by hand for 5 s, add 6 mL n-hexane:isopropanol 5:1, rotate on a tumble-mixer at 28 rpm for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 120 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:20 mM pH 6 sodium phosphate buffer 15:40:45
Flow rate: 1.2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 9.2
Internal standard: 1-pentyl-2-(2',6'-xylylcarbamoyl)piperidine hydrochloride (13.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: fentanyl, morphine, oxycodone

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kastrissios,H.; Hung,M.-F.; Triggs,E.J. High-performance liquid chromatographic method for the quantitation of bupivacaine, 2,6-pipecoloxylidide and 4'-hydroxybupivacaine in plasma and urine, *J.Chromatogr.*, **1992**, *577*, 103–107.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 10 μL 100 μg/mL etidocaine, add to a 3 mL Extrelut SPE cartridge, elute with

15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 11
Internal standard: etidocaine (14)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine, prilocaine, dibucaine
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 5% bupivacaine hydrochloride injection and 25 mg/mL morphine sulfate injection with 0.9% NaCl injections to a bupivacaine concentration of 625 μg/mL and a morphine concentration of 100 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:100 mM pH 5 phosphate buffer 40:60
Flow rate: 1.5
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: morphine

KEY WORDS
injections; stability indicating

REFERENCE
Johnson,C.E.; Christen,C.; Perez,M.M.; Ma,M. Compatibility of bupivacaine hydrochloride and morphine sulfate, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 61–64.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot

HPLC VARIABLES
Column: 100 × 3.9 4 μm Radial Pak phenyl (Waters)
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 phosphate buffer containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: degradation products, fentanyl

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Tu,Y.H.; Stiles,M.L.; Allen,L.V.,Jr. Stability of fentanyl citrate and bupivacaine hydrochloride in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1990**, *47*, 2037–2040.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere XL-ODS C18
Mobile phase: MeCN:100 mM pH 5 KH_2PO_4 40:60
Flow rate: 1.5
Detector: UV 254

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Jones,J.W.; Davis,A.T. Stability of bupivacaine hydrochloride in polypropylene syringes, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2364–2365.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:20 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 14.1
Limit of detection: 254 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, hydromorphone

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:0.092%phosphoric acid + 0.2% triethylamine 26:74
Flow rate: 2
Detector: UV 214

CHROMATOGRAM
Internal standard: lidocaine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Also analyzed: diphenhydramine, diltiazem, metabolites

KEY WORDS
rat; liver

REFERENCE
Hussain,M.D.; Tam,Y.K.; Gray,M.R.; Coutts,K.T. Kinetic interactions of lidocaine, diphenhydramine, and verapamil with diltiazem: A study using isolated perfused rat liver, *Drug Metab.Dispos.*, **1994**, *22*, 530–536.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlor-

promazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: butacaine, lidocaine, benzocaine, tetracaine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH:isopropanol 90:10 containing 15 mM N-benzyloxycarbonylglycyl-L-proline and 9 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: k' 3.09 (first enantiomer)

KEY WORDS
chiral; α = 1.08

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 10.60 (A), 5.79 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, le-

vorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.